Studies on theobromine disposition in normal subjects

Alterations induced by dietary abstention from or exposure to methylxanthines

Normal male volunteers on xanthine-restricted diets had a plasma theobromine (TB) half-life (t½) of 6.1 ± 0.7 hr (mean ± SEM), a TB metabolic clearance rate (MCR) of 113.8 ± 6.8 ml/min (mean ± SEM), and an apparent volume of distribution (aVd) of 59.9 ± 7.8 L (mean ± SEM) after a single oral dose of 6 mg/kg of TB. A two-week period of dietary abstention from methylxanthines shortened the mean plasma TB t½ by 33% and increased the mean MCR of TB by 59%; no change occurred in mean aVd of TB. In another experiment, normal male volunteers received the same oral dose of TB for each of five consecutive days. The mean plasma TB t½ measured immediately after the last TB dose was prolonged by almost two-thirds, while the mean aVd increased 23%. Although the mean plasma TB t½ measured four days after this last TB dose was the same as that measured after a single oral dose, the mean MCR of TB increased by 34%, but this change was balanced by increased mean aVd of 35%. These results suggest that immediately after five daily doses of TB, an impairment of TB clearance occurs that is reversible by four days of dietary abstention from methylxanthines.

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Since many people ingest theobromine (TB) in the form of chocolate, coffee, tea, and various cola beverages, TB concentrations in certain tissues of these subjects may be high. Such individuals might possibly experience drug interactions involving TB when drugs are administered or on exposure to the other methylxanthines, theophylline and caffeine. No such interactions have been reported. The present paper describes interactions that involve only TB and reports TB disposition following intravenous TB administration, two weeks after abstinence from all methylxanthines and immediately after five daily doses of TB as well as four days after the last of the five daily doses of TB.

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Materials and methods

Materials. The TB used in this study was in the form of crystals obtained from Sigma Chemical Co.

Subjects. Nonmedicated, normal male volunteers 21 to 29 yr of age were divided into two groups. Following two days of abstinence from all methylxanthines, each of the 6 subjects in

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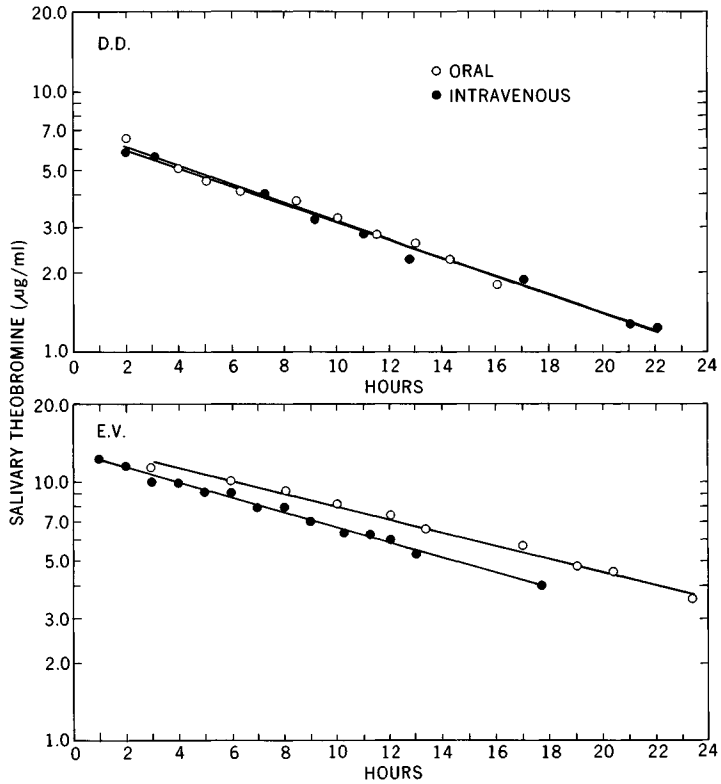

Fig. 1. Decay of methylxanthine in saliva of two normal subjects after a single oral dose of TB (6 mg/kg) followed four days later by the same dose of TB administered intravenously. Note that the β phase of the TB salivary decay curve in each subject is similar for the oral and intravenous routes. The extrapolated concentration at zero time for D. D. was 7.2 μg/ml after oral administration and 6.9 μg/ml after intravenous administration; for E. V., the values were 14.2 and 12.7 μg/ml, respectively.

Group 1 received a single oral dose of TB (6 mg/kg) in aqueous suspension after an overnight fast. No food was taken for at least 2 hr after TB administration. For determination of plasma and salivary TB decay curves, blood and saliva specimens were collected from each of the 6 subjects at 6, 8, 10, 12, 14, 16, and 18 hr after TB administration. During the following two weeks, each subject maintained strict dietary abstinence from all methylxanthines. On the fifteenth day after the first TB dose, another dose of TB (6 mg/kg) was administered orally. Saliva samples were collected following the same schedule as before to determine TB decay rates.

The 9 subjects in Group 2 received a single oral dose of TB (6 mg/kg). Blood and saliva specimens were collected at 6, 8, 10, 12, 14, 16, and 18 hr after TB administration. The same TB dose of 6 mg/kg was also given orally in aqueous suspension on each of the four successive mornings. On the fifth day of TB administration, saliva samples were collected on the same schedule as after the first dose of TB. On the ninth day after the first dose of TB, a sixth dose of TB (6 mg/kg) was administered orally and saliva samples collected as above for determination of TB decay rates.

Two normal subjects received an intravenous dose of TB (6 mg/kg) in one liter of 5% dextrose and water administered as a slow drip over two hours. Saliva samples were collected as before for determination of TB decay rates. Four days later, the same subjects received a single oral dose of TB (6 mg/kg) completely dissolved in tap water. Saliva samples were collected as before.

Each subject abstained from all methylxan-

Table I. *Effect of dietary abstinence from methylxanthines on theobromine disposition*

	Group 1		*Half-life (hr)*			
Subject	*Age*	*Wt (kg)*	*After single dose*	*Two weeks' abstention*	*(% change)*	*After single dose*
R. M.	21	93.2	7.9	4.1	(−48%)	39.6
J. B.	25	79.5	5.5	3.8	(−31%)	45.7
S. S.	28	72.7	11.5	7.4	(−36%)	51.4
J. N.	28	65.9	8.8	5.7	(−35%)	59.9
A. G.	25	102.3	9.1	8.0	(−12%)	61.6
M. S.	29	63.6	11.8	7.5	(−36%)	70.0
Mean ± SEM			9.1 ± 0.9	6.1 ± 0.7	(−33%)	54.7 ± 4.6
p value				<0.005		

Table II. *Alterations in theobromine disposition produced by subacute theobromine administration*

	Group 2		*Half-life (hr)*			
Subject	*Age*	*Wt (kg)*	*After single dose*	*Immediately after fifth dose (% change from single dose)*	*Four days after fifth dose of TB (% change from single dose)*	*After single dose*
P. B.	23	84.1	8.8	24.0 (+173%)	7.6 (−14%)	41.5
M. K.	24	68.2	6.6	8.1 (+ 23%)	7.3 (+11%)	40.2
J. K.	23	61.0	9.1	7.7 (− 15%)	6.6 (−27%)	48.5
G. P.	24	46.0	8.0	18.7 (+134%)	6.8 (−15%)	34.2
D. K.	24	61.0	8.1	10.7 (+ 32%)	7.4 (− 9%)	36.6
L. L.	26	62.7	5.5	15.1 (+174%)	9.7 (+76%)	25.5
J. S.	24	69.0	8.8	8.2 (− 7%)	8.9 (+ 1%)	39.4
B. B.	29	71.4	6.6	9.3 (+ 41%)	7.5 (+14%)	46.7
J. T.	25	70.0	6.2	8.3 (+ 34%)	6.2 (No Δ)	41.7
Mean ± SEM			7.5 ± 0.4	12.2 ± 1.9 (+ 63%)	7.6 ± 0.4 (+ 1%)	39.3 ± 2.3
p value				<0.025*	>>0.1†	

*Compares value after single dose to value after the fifth daily dose.

†Compares value after single dose to value four days after the fifth daily dose.

thines during the course of this study beginning three days prior to the first TB dose. An overnight fast preceded each oral TB dose: no food was taken for at least two hours after TB administration.

Assay procedure. TB concentrations in saliva and plasma were measured by reverse-phase high-pressure liquid chromatography (HPLC) according to Evanson and Warren.[3] A Waters Associates, high-performance liquid chromatography, ALC GPC 244 system with a μ Bond-a-Pac C-18 column, was used for all TB determinations. The liquid phase for the HPLC consisted of 10% acetonitrile in 0.01 M acetate buffer at pH 4.0. The flow rate was 2 ml/min. Ultraviolet detection was at 254 nm. Retention times for TB and the internal standard, β-hydroxypropyl theophylline, were 2.3 min and 3.8 min, respectively. A standard curve constructed with TB concentrations of 2.5, 3, 5, 8, and 10 μg/ml yielded a straight line that passed through the origin and all five points obtained at these concentrations. These standard TB concentrations were prepared in blank plasma and saliva; the blanks read 0. Peak height ratios were plotted against TB concentration.

Pharmacokinetic parameters. The apparent volume of distribution (aVd) of theobromine was calculated from the administered dose and

Apparent volume of distribution (L)		*Metabolic clearance rate (ml/min)*		
Two weeks' abstention	*(% change)*	*After single dose*	*Two weeks' abstention*	*(% change)*
33.4	(−16%)	57.7	93.0	(+61%)
46.5	(+ 2%)	95.4	141.4	(+48%)
76.8	(+49%)	51.4	119.2	(+132%)
49.8	(−17%)	78.8	101.4	(+29%)
79.6	(+29%)	78.4	115.4	(+47%)
73.3	(+ 5%)	68.7	112.1	(+63%)
59.9 ± 7.8	(+ 9%)	71.7 ± 6.5	113.8 ± 6.8	(+59%)
>0.1			<0.05	

Apparent volume of distribution (L)		*Metabolic clearance rate (ml/min)*		
Immediately after fifth dose (% change from single dose)	*Four days after fifth dose of TB (% change from single dose)*	*After single dose*	*Immediately after fifth dose (% change from single dose)*	*Four days after fifth dose of TB (% change from single dose)*
48.9 (+ 18%)	48.3 (+ 16%)	54.2	23.5 (−57%)	73.3 (+ 35%)
49.8 (+ 24%)	56.3 (+ 40%)	70.7	71.3 (+ 1%)	89.0 (+ 26%)
52.4 (+ 8%)	74.0 (+ 53%)	61.8	78.7 (+27%)	129.4 (+109%)
44.4 (+ 30%)	35.0 (+ 2%)	49.5	27.4 (−45%)	59.1 (+ 19%)
51.0 (+ 39%)	46.7 (+ 28%)	52.4	55.1 (+ 5%)	72.5 (+ 38%)
59.4 (+133%)	55.7 (+118%)	53.9	45.5 (−16%)	66.1 (+ 23%)
25.9 (− 34%)	48.6 (+ 23%)	51.8	36.4 (−30%)	63.4 (+ 22%)
64.6 (+ 38%)	62.0 (+ 33%)	82.0	80.1 (− 2%)	95.2 (+ 16%)
40.7 (− 2%)	50.4 (+ 21%)	77.9	56.2 (−28%)	94.1 (+ 21%)
48.5 ± 3.7 (+ 23%)	53.0 ± 3.6 (+ 35%)	61.6 ± 4.1	52.7 ± 7.1 (−14%)	82.5 ± 7.4 (+ 34%)
<0.05*	<0.005†		<0.05*	<0.005†

the extrapolated zero time concentration (i. e., B). The aVd is therefore equivalent to (Vd) extrapolated and

$$aVd = DOSE/B \quad (1)$$

instead of the more rigorous equation

$$aVd = DOSE/(AUC)\beta \quad (2)$$

where AUC is the area under the plasma concentration-time curve and β is the slope of the terminal linear phase (i.e., $0.693/t_{1/2}$). Preliminary studies in 2 normal subjects indicate that calculation of aVd using Equation 1 instead of Equation 2 introduces an error of not more than 7%.

The metabolic clearance rate (MCR) was determined from the equation

$$MCR = aVd(\beta) = k_{el}(Vd)_{cc} \quad (3)$$

where k_{el} is the apparent first-order rate constant for elimination of TB from the body and $(Vd)_{cc}$ is the apparent volume of distribution of the central compartment.

Results

In the same individual, TB half-lives ($t_{1/2}$) in plasma and saliva were not significantly different ($p < 0.1$, paired t test). The mean ratio ± SE for saliva to plasma TB concentrations was 0.96 ± 0.19. Fig. 1 shows that regardless of whether TB is administered to an

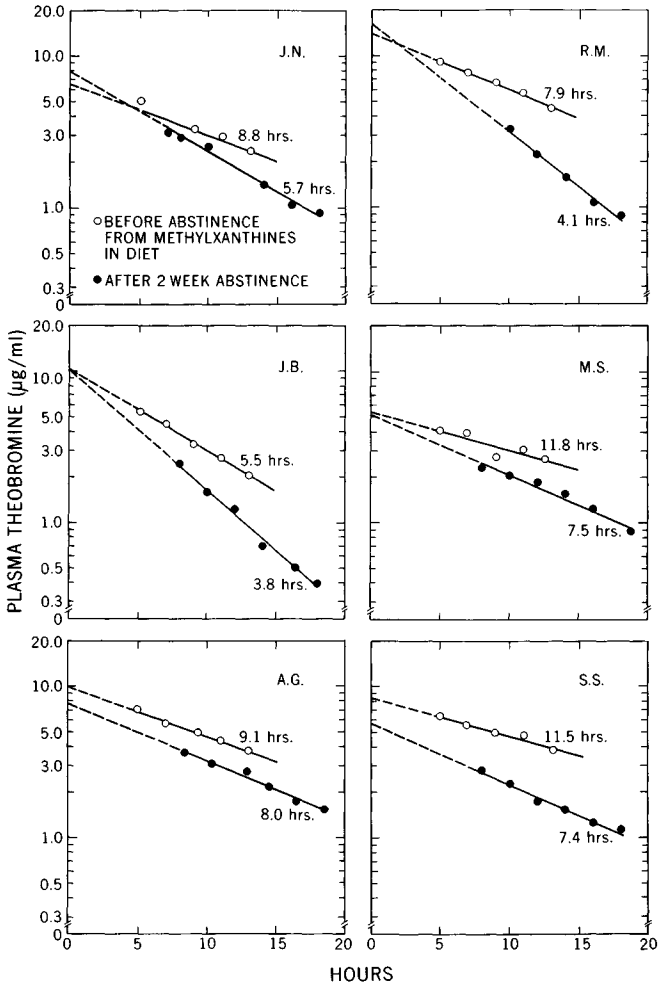

Fig. 2. Decay of a single oral dose of TB (6 mg/kg) before and after a two-week dietary abstention from methylxanthines.

individual orally or intravenously, the β phase of TB decay in saliva and the extrapolated zero time concentration are not significantly altered by the choice of route. The data in Fig. 1 indicate that salivary TB concentrations were similar beginning at 2 hr and continuing for 18 to 22 hr after either intravenous or oral administration, suggesting that TB in the aqueous solution in which we gave it was completely absorbed from the gastrointestinal tract. This experiment also reveals that, although approximately twofold interindividual differences exist in TB t½s as well as in TB MCR of 6 normal subjects (Table I), intraindividual variations are exceedingly small (Fig. 1). Thus, a subject's pharmacokinetic values for TB disposition can be closely reproduced when the same dose of TB is readministered four days after the first dose (Fig. 1).

Abstention from all methylxanthines for 2 wk decreased the plasma TB t½ ($p < 0.005$) and increased the MCR of TB ($p < 0.005$) after a single oral dose of TB (6 mg/kg) (Table I, Fig. 2). The aVd of TB did not change (Table I, Fig. 2).

Daily ingestion of TB (6 mg/kg for five days) produced significant alterations in TB disposition when t½, aVd, and MCR were measured after the fifth daily TB dose. Table II showed increased TB t½ ($p < 0.025$) and aVd

($p < 0.05$), whereas the MCR of TB decreased ($p < 0.05$). Measurements made four days after the last of the five daily TB doses indicated return of the mean TB t½ in saliva to the value obtained after a single oral dose (Table II). Compared to the single oral dose, however (Table II), the mean aVd was increased by 35%, as was the mean MCR ($p < 0.005$).

Discussion

In the United States, TB is a constituent of such dietary staples as chocolate, coffee, tea, and many colas; the oral dose of TB selected for the present study (6 mg/kg) is equivalent to that consumed in five cups of hot chocolate. Our paper provides pharmacokinetic measurements of TB in normal subjects and describes effects on these values of dietary abstention from methylxanthines or of high daily TB intake.

The fact that in normal volunteers a two-week dietary abstention from methylxanthines accelerated the mean plasma TB t½ by 33% and increased the mean MCR of TB by 59% without altering the aVd suggests that normal dietary exposure to TB may produce sufficiently high tissue concentrations of TB, its metabolites, or both, to retard TB elimination, possibly by inhibiting an enzyme system responsible for TB metabolism. It should be emphasized that these changes in TB t½ and MCR are expressed as means, explaining why the increase in mean MCR is not balanced perfectly by alterations in mean half-life. Further evidence for the retarding effects exerted by TB administration on its own elimination rate comes from our experiment comparing TB disposition before and after five daily doses of TB. Immediately after the fifth dose of TB, the mean TB t½ and aVd increased by 63% and 23%, respectively, whereas the mean TB MCR decreased by 14%. Since we were unable to obtain complete pharmacokinetic measurements during the earliest times in this study, we cannot obtain the pharmacokinetic parameters k_{el} and $(Vd)_{cc}$ and therefore MCR was calculated from the product of aVd and β. Thus, we cannot determine whether reduction of mean TB MCR by 16% arises from impaired TB metabolism, a decrease in $(Vd)_{cc}$, or a combination of these events.

Impairment of TB clearance immediately after the fifth daily dose of TB seems to vanish four days later as indicated by a return of mean TB t½s to values obtained prior to subacute TB administration and by a 34% increase in TB MCR. This change in MCR is almost exactly balanced by the 35% increase in aVd (Table II). It is uncertain whether this change in aVd represents a true increase in the volume of distribution of TB or simply reduced gastrointestinal absorption attributable to chronic mucosal irritation after subacute TB administration. An effect due entirely to chronic mucosal irritation seems unlikely because the effect is greater four days after discontinuation of TB than immediately after the fifth dose of TB. Reduced gastrointestinal absorption of TB could also arise from induction of TB-metabolizing enzymes in the gut by five daily doses of TB.

In these subacute experiments, TB was not administered intravenously due to the unknown cardiovascular toxicity of TB by this route; therefore, the oral route had to be employed. In the subacute experiments, both aVd and MCR may differ after oral and intravenous TB administration, although after single oral or intravenous doses of TB, our experiments revealed in an individual similar values for β or for the extrapolated zero time concentration, regardless of route (Fig. 1). For the purpose of the present study, pharmacokinetic parameters are compared in an individual only after oral TB administration in order to determine what changes subacute oral TB administration, the route of TB consumption in practice, produces.

This experiment illustrates the critical role played by the particular time selected for analysis of drug interactions and the importance of assessing drug interactions at more than a single point in time. If the study had stopped immediately after the fifth dose of TB, the five daily TB doses would seem to have produced only an inhibitory effect on TB elimination. However, by examining a time point four days beyond the last TB dose, we observed an increase in TB clearance. An earlier study on quinine-induced alterations in both quinine and antipyrine disposition suggested that an inductive effect of quinine on antipyrine disposition was concealed while appreciable quinine con-

centrations were present, but appeared when the interaction was investigated later in time to permit quinine elimination from plasma.[1]

The literature on drug interactions is immense and frequently conflicting; some discrepancies probably arise from failure to recognize the critical role that timing of observations can play in determining qualitatively and quantitatively the character of the interaction. In the present experiments on TB and in the previous ones on quinine, we observed a dual effect that depended on the time at which the observations were made.

In man the enzyme system responsible for TB catabolism and its location are unknown. Theophylline metabolism in the rat has been shown to occur in hepatic smooth endoplasmic reticulum.[5] Induction of cytochrome P-448 by 3-methylcholanthrene administration accelerated the decay of ^{14}C-theophylline. Burg and Werner[2] reported that hepatic microsomes and the renal cytosol-microsomal fraction failed to demethylate TB or caffeine.

Theophylline is frequently prescribed in the treatment of asthma to relax respiratory smooth muscle. Therapeutic theophylline concentrations in plasma approximate 10 ml/L; concentrations in excess of 20 ml/L can be toxic.[4, 7, 8] Both TB and caffeine inhibit theophylline metabolism in rat liver slices.[5] The possibility that high plasma methylxanthine concentrations may alter theophylline disposition, thereby contributing to theophylline accumulation and toxicity, should be explored.

During the preparation of this manuscript, an abstract[6] appeared in which pharmacokinetic data on TB elimination in breast-feeding mothers were given. The values in that abstract closely resembled those obtained in the present study although the dose of TB administered was not precise (all six postpartum mothers receiving 4 oz of milk chocolate which was estimated to contain approximately 240 mg of TB).

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